

SCIENTIFIC LETTER

Interstitial fibrosis in the dilated non-ischaemic myocardium

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Coronary artery occlusion and a variety of inflammatory disorders are followed by focal or segmental areas of fibrous scarring. The causes of interstitial fibrosis are less clear. Variable but sometimes dense interstitial fibrosis is seen in dilated cardiomyopathy.¹ Some reports suggest that interstitial fibrosis is also increased in non-infarcted myocardium from hearts with ischaemic scars.² Because of current interest in the pathophysiology of ventricular dilatation, we compared the interstitial collagen content of the left and right ventricles in dilated and undilated hearts of subjects without significant coronary artery occlusion. Our hypothesis was that we would find histological evidence of increased interstitial collagen content in dilated hearts.

METHODS

Forty four hearts were selected from approximately 500 necropsies of adults who died in the community or at Southampton University Hospitals over a six month period. For case selection mitral valve circumference was measured. Hearts with a mitral valve circumference > 110 mm were provisionally assigned to the test (dilated) group and those < 110 mm to the control group. Five criteria were then considered. If any of these applied to the hearts from either the test or the control group, they were excluded from further study. These were (1) a clinical history of ischaemic heart disease; (2) macroscopic evidence of old or recent ischaemic heart disease; (3) any stenosis > 50% of the external diameter of a major epicardial coronary artery; (4) valvar or congenital cardiac disease apart from mild thickening of the anterior mitral valve leaflet; and (5) treatment with angiotensin converting enzyme inhibitors. In the selected hearts the following were measured: mitral valve circumference (mm), cutting the chordae tendineae to facilitate straightening of the valve rim, aortic valve circumference (mm), thickness of the free wall of the left ventricle and the interventricular septum 10 mm below the mitral valve ring (mm), thickness of the interventricular septum at the base of the papillary muscles (mm), thickness of the free wall of the right ventricle 20 mm below the pulmonary valve (mm), the distance from the apex of the left ventricle to the base of the mitral valve annulus (mm), and the weights of the right ventricle, left ventricle, and interventricular septum (g). To estimate left ventricular volume (cm³) we used an equation that assumes that the cavity is conical in shape, $0.83(Ax)$, where A is the area of the mitral valve annulus (cm²) and x the distance from the mitral valve annulus to the apex of the left ventricle (cm).

Paraffin sections (5 µm) of the interventricular septum and the free walls of the left and right ventricles were stained with sirius red F3B, which specifically stains all types of fibrillar collagen. The sections were pretreated with 0.2% phosphomolybdic acid for five minutes and then stained for 90 minutes in 0.1% sirius red in saturated aqueous picric acid. Sections were then treated with 0.01 M hydrochloric acid for two minutes, dehydrated, and mounted. Sirius red staining was measured with an interactive computerised image analysis

system (Macintosh hardware, Colour Vision 1.7 software). A density slice of the sirius red positive staining area was produced and expressed as a percentage of the area of the field. For each of the three regions measured in each heart, the mean of 20 high power fields (× 400) was calculated. Because there is a network of collagen bundles extending from intramyocardial vessels to cardiac myocytes in all regions of the normal heart, we avoided perivascular areas during image analysis.

RESULTS

Table 1 summarises the clinical features of the patients and their causes of death. The groups of patients were well matched for age but men predominated in the dilated group

Table 1 Features of patients in each study group and postmortem measurements

Feature	Dilated (n=22)	Control (n=22)
Men: women	14:8	7:15
Mean age (years) (range)	71.8 (44–96)	68.6 (30–97)
Cardiac medications (number of patients receiving treatment)		
β Blockers	0	2
Diuretics	7	5
Ca ²⁺ channel blockers	1	3
Antiarrhythmics	1	2
Digoxin	3	1
Cause of death		
Respiratory tract infection	6	3
Chronic obstructive airways disease	3	0
Pulmonary embolism	3	3
Ruptured aortic aneurysm	2	1
Cerebrovascular accident	2	4
Road traffic accident	2	2
Carcinoma	0	3
Gastrointestinal disease	3	2
Suicide	1	1
Others†	0	3
Heart weight (g)		
Whole heart mass	426.2 (16.6)*	351.6 (15.3)
Whole ventricular mass	328.5 (21.7)	268.6 (13.1)
Left ventricle	239.3 (12.6)*	183.8 (9.7)
Right ventricle	84.6 (4.2)	70.3 (3.9)
Valve circumferences (cm)		
Aortic valve	8.08 (0.22)	7.42 (0.17)
Mitral valve	12.12 (0.18)*	9.78 (0.17)
Ventricular thickness (cm)		
Left ventricle	1.72 (0.05)	1.55 (0.06)
Interventricular septum 1	1.80 (0.05)	1.62 (0.06)
Interventricular septum 2	1.81 (0.05)	1.60 (0.06)
Right ventricle	0.57 (0.03)	0.50 (0.03)
LV volume (cm ³)	83.5 (4.7)*	51.2 (2.7)
LV mass: LV volume (g/cm ³)	2.86*	3.59
Collagen proportion (%)		
Left ventricle	6.14 (0.20)*	3.76 (0.12)
Interventricular septum	6.25 (0.22)*	3.70 (0.10)
Right ventricle	4.53 (0.14)*	3.60 (0.10)

*p<0.01. Values are mean (SEM).

†Septicaemia, cardiac arrhythmia, hepatic failure.
LV, left ventricular.

and women in the control group. There was no clear difference in the causes of death or the use of cardiac drugs between the groups. Eight patients in the dilated group and 10 in the control group were taking medications that can have a hypotensive effect. The circumference of the mitral valve annulus was significantly greater in the dilated group (mean 12.12 cm v 9.78 cm, $p < 0.01$) but there was no significant difference in the aortic valve circumference. Total heart weight, left ventricular weight, and left ventricular volume were significantly greater in the dilated group. There were no consistent differences in ventricular thickness between the groups. There was a close relation between left ventricular volume and left ventricular weight in the dilated but not in the control group. The ratio of left ventricular mass to left ventricular volume was significantly reduced in the dilated as compared with the control group. There was no macroscopic or histological evidence of cardiomyopathy or myocarditis in any of the patients studied. The proportion of collagen as determined by sirius red staining was significantly greater in the dilated group in all three areas that were studied. In the free wall of the left ventricle and in the interventricular septum this difference was substantial. In the dilated group the proportion of collagen in both the left ventricle and septum was significantly greater than in the right ventricle ($p < 0.01$). Linear regression analysis showed that there was no relation between the proportion of interstitial collagen in any of the three regions examined histologically and either the age or the sex of the patients studied or the whole or individual ventricular weights.

DISCUSSION

These findings confirm our hypothesis that interstitial collagen content is increased in hearts with dilated left ventricles but no clinical or pathological evidence of ischaemic heart disease. Similar results have been reported in patients with dilated cardiomyopathy,¹ in experimental models of systemic hypertension³ and, in some² but not all⁴ reports, in the residual non-infarcted myocardium of ischaemic hearts. The strength of our study is the substantial difference that we observed between groups in all areas of the left and right ventricle that we studied. The lack of clinical information about some patients was an important limitation of our study. We cannot exclude the possibility that some patients had undiagnosed or insufficiently treated systemic hypertension or disease of small epicardial arteries or intramyocardial vessels. We were careful to exclude patients with a definite clinical history of cardiac

failure or evidence of this at postmortem examination. It is possible that some patients had myocarditis, but we suggest that in the majority of cases there would be evidence of this in at least one of the three histological blocks that were examined.

There are several other limitations. The formula that we used for estimating left ventricular volume produced values that were lower than in living patients. The use of a mitral valve circumference of 110 mm to distinguish normal from dilated left ventricles has not been previously tested, although it is more than 10 mm greater than the mean plus 95% confidence interval quoted in one study of normal mitral valves.⁵ In addition to collagen, sirius red stains amyloid and some inflammatory cells but we did not suspect amyloidosis in any of the cases we examined.

In conclusion, unexplained ventricular dilatation is present in about 4% of postmortem examinations. Whole heart weight and left ventricular weight in these patients were significantly greater than in patients with undilated ventricles and no evidence of cardiac disease. A histological method showed that there was a significant increase in interstitial collagen in the free wall of the left and right ventricles and the interventricular septum.

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